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RECENT STUDIES ON CHEMICAL MEDIATIONS OF NERVE IMPULSES*

W. B. CANNON
From the Laboratories of Physiology of the Harvard Medical School
BOSTON

The chemical mediation of nerve impulses by the action of adrenin is well known. Any general stimulation of the sympathetic division of the autonomic nervous system causes a secretion of adrenin, and this adrenin, widely distributed by the blood, has as a rule the same effects as the sympathetic impulses. Thus a viscus, even though deprived of its sympathetic nerves, may be brought into action with the other viscera because adrenin acts as an intermediary between the responsive organ and the nerve impulses which come to the adrenal medulla.

Recent studies have shown that the action of adrenin, as an intermediary agent bringing about effects in the organism equivalent to those caused by nerve impulses, illustrates an extensive phenomenon of such mediation. These recent researches, however, have been concerned with a much more intimate relation between the impulses and the reacting tissues. Indeed, they show that chemical substances are set free by the impulses near or within the cells which make up the tissue. The evidence for this phenomenon was first presented by Loewi (1) in 1921 when he reported that Ringer's solution, which had been in contact with a frog heart subjected to vagus influence, acquired a new property, that of bringing about in another frog heart the inhibitory effect which is characteristic of vagus stimulation. He also showed that if the first heart was stimulated through the sympathetic fibres, the Ringer's solution applied to a second heart would undergo the acceleration characteristic of sympathetic influences. These fundamental observations have been confirmed by numerous investigators and the phenomenon has been observed not only on frog and tortoise hearts but also on the heart of warm-blooded animals (2). Furthermore, it has been reported that the material given off from the heart has typical vagal and sympathetic effects on the stomach and intestine (3). Since the experimental procedure is difficult, there have been, naturally enough, reports of failure to obtain the results described by Loewi and the others who have confirmed his work.

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Another forward step was taken last year when Finkelman (4) reported that if Ringer's solution is allowed to run over a pulsating piece of rabbit intestine, A, still supplied with its mesenteric nerves, and pass thence to another piece of pulsating intestine, B, the solution acquires a new property when the nerves of piece A are stimulated—piece A is inhibited and thereby the piece B is inhibited also.

Furthermore, Dale and Gaddum (5) have recently reviewed, repeated and extended the observations of Vulpian (6) and of Sherrington (7) on the curious, slow contraction of denervated skeletal muscle when neighboring parasympathetic nerves are stimulated, and they have offered evidence that the phenomenon can reasonably be explained as due to the effect on the muscle of a chemical substance locally discharged by parasympathetic impulses.

From the foregoing observations it is evident that peculiar substances are given off from or near effector organs innervated by sympathetic and parasympathetic fibres of the autonomic nervous system and that these substances are capable of influencing other organs, similarly innervated, just as the nerve impulses do. Examination of the "vagus stuff," as Loewi calls it, shows that it is dialyzable, insensitive to acid, very sensitive to alkali, and that its activity can be readily destroyed by treatment with a tissue esterase (8). In general, the evidence points directly towards its being a highly unstable and very active ester of choline and possibly acetyl-choline itself. Although it remains stable for a long time in the undisturbed heart, it is rapidly broken down after it is once freed by vagus stimulation or by mincing cardiac tissue (9). In 1901, Hunt (10) found in aqueous extracts of the adrenal gland a blood-pressure reducing substance which on chemical treatment disappeared as the choline content increased. He inferred that in the extracts there was an ester-like "precursor" of choline, and this led him to the synthesis of acetyl-choline and to his later classical studies of the pharmacology of this substance. Dale and Dudley (11) have recently been able to isolate and identify acetyl-choline in extracts of fresh spleen. The high degree of instability of acetyl-choline probably accounts for the failure to identify the substance in tissues until recently.

The accelerator substance, derived from cardiac tissue subjected to sympathetic stimulation, is destroyed when the cardiac contents are ashed. Like adrenin, it is rendered inactive when mixed with eosin and exposed to ultraviolet light (12). Like adrenin, also, the solution from the stimulated heart causes constriction of the frog's arterioles. Like adrenin, it is rendered more effective by a dilute solution of glycocoll, and like adrenin it loses its activity on standing in air for 20 hours or on being heated to 100° C. (13).

It is interesting to observe that the two substances mentioned as being produced by parasympathetic and sympathetic nerve impulses, acetyl-choline and adrenin, respectively, have, when injected into the organism,

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the usual effects which are produced by the nerve impulses. If we consider that these substances are set free by the nerve impulses as they come to the effector cells, we may regard the substances as chemical mediators of the impulses.

The point has been made by Demoor that while the phenomena which have been briefly described above are true for isolated cardiac muscle and intestine bathed in Ringer's solution, or at best in a mixture of Ringer's solution and defibrinated blood, there is no clear proof that the phenomena occur under natural conditions. Although arguing for the reality of the humoral transmission of nerve impulses, Demoor has declared, "it is evident that the irrigations of the heart which are a part of all these investigations may create new conditions of existence for the tissues, conditions accompanied by permeabilities which do not exist in physiological states, and that the escape of vagal and sympathetic substances, though proved experimentally, may not occur normally." Evidence that this wise precaution on the part of Demoor may be now set aside, at least with respect to the sympathicomimetic substance, has recently been obtained at the Harvard Physiological Laboratories (15, 16) and I propose now to present that evidence.

For many years the heart isolated from the central nervous system—the "denervated" heart—has been used by us as an indicator of chemical changes occurring in the blood stream. This preparation is very insensitive to alterations of blood pressure, but highly sensitive to circulating adrenin. It will respond with a faster beat to a dilution of adrenin 1 part in 1,400,000,000 parts of blood (17). By means of this delicate indicator, studies have been conducted on the effect of cold, asphyxia, motion and emotion, and other conditions, on secretion from the adrenal medulla.

Although the immediate effect of emotional excitement, for example, disappears when the adrenal glands are rendered inactive by removal of one and denervation of the other, it has been known for some years that there is a slow increase of heart rate which, unlike the quick adrenin effect, reaches its maximum about three minutes after the brief excitement ceases. This curious phenomenon has presented to workers in the laboratory a tantalizing and mysterious problem, and much time and experimentation have been spent in attempts to explain it. The phenomenon continued after exclusion of all known or possible cardio-accelerator agencies. It continued after removal of one adrenal and the medulla of the other, after extirpation of the accessory chromophil substance on the front of the aorta, after denervation of the pancreas and intestinal mucosa, after removal of the pituitary body, the thyroids and the parathyroids, as well as the gonads. When the upper thoracic sympathetic chains had been removed and the two abdominal chains, as well, the phenomenon still persisted. It disappeared on excision of the remnants of the sympathetic in the lower chest and it remained in abeyance for many weeks thereafter. From this observation it was clear that a substance is probably given off into the

blood stream when structures innervated by the sympathetic system are stimulated—a substance which has the accelerating action on the denervated heart that is characteristic of circulating adrenin.

Experimental proof of the actual existence of a circulating mediator of widely distributed sympathetic impulses was difficult to obtain because the anesthetics which were tried abolished the effect of stimuli which might bring the sympathetic system into action. Finally proof was obtained, however, in a preparation in which the heart was denervated, the adrenals and liver excluded from action, and the spinal cord severed in the mid-thoracic region (so as to permit without anesthesia painless operations in the hind part of the animal). With this preparation a stimulation of the sympathetic fibres distributed to the smooth muscles of the tail hairs caused a slow increase of blood pressure and heart rate, reaching a maximum in two or three minutes and gradually returning to the former level. More recently we have found that the anesthetic, "Dial," can be used to produce complete anesthesia without abolishing the phenomenon of remote action of sympathetic influence.

That there really is a substance given off into the blood stream when smooth muscle is subjected to sympathetic control has been proved by checking the blood flow into and from the tail region during the stimulation and then allowing the flow to continue. The rise of blood pressure and the acceleration of the heart are then much reduced or are delayed until the flow is restored. Further proof has lately been obtained by stimulating a part of the lower abdominal sympathetic which causes erection of the hairs in a circumscribed patch of the back of the animal. When this area is removed, precisely the same stimulus as before has no effect.

Since the substance which accelerates the denervated heart—and to this may be added the denervated submaxillary gland and the denervated nictitating membrane—is derived from smooth muscles when they are influenced by sympathetic impulses, we have suggested that it be called "sympathin." Quite possibly sympathin is the same substance as adrenin, but as it has a different source, it may be justifiably given a different name.

In this connection it is of interest to note that cocaine, which greatly sensitizes the smooth muscle of blood vessels to the action of adrenin, has a similar effect on sympathin (18). Quantitation of sympathin in terms of adrenin shows that the total amount of sympathin produced as a consequence of stimulating the tail hairs for a half-minute is equivalent to 0.000005 gram of adrenin.

The two substances, sympathin and adrenin, Rosenblueth and I have recently shown to be coöperative. If the denervated nictitating membrane is used as an indicator and a threshold stimulus for medulliadrenal secretion and sympathin secretion has been established, the consequence of causing a discharge of both sympathin and adrenin with such stimuli is a greatly augmented contraction of the membrane.

The demonstration that sympathin, resulting from the action of sympathetic impulses on smooth muscle, is given off in sufficient quantity to affect through the blood stream distant structures, suggested that by properly contrived experiments the "vagus stuff" could be similarly demonstrated. Freeman, Phillips and I (19), using the completely denervated iris and submaxillary gland, and also the blood pressure, the denervated heart (with intrinsic vagal neurones present) and the denervated tongue, as indicators, tested the effect of stimulating the whole vagal distribution below the cardiac branches. Although we arranged a by-pass for the blood from the portal vein to the inferior cava in order to avoid possible destruction of the vagus substance by the liver, and although we employed various anesthetics and in some experiments excluded the need for anesthesia, all our results were negative. It would appear, therefore, that the influence of the parasympathetic substance is quite local. This conclusion is in harmony with the view that as a rule the parasympathetic nerves distribute directly to the structures innervated and thus are organized for specific innervation of those structures rather than for diffuse effects such as are produced by the sympathetic impulses in coöperation with adrenin and sympathin.

About ten years ago experiments were reported that demonstrated the production of a substance by the liver, when under sympathetic influence, that caused acceleration of the denervated heart (20). The acceleration of the heart produced by sympathin raised the question as to whether the cardio-accelerator substance from the liver might not also be sympathin. This question has not yet been definitely settled. It appears that the liver substance is augmented by eating protein food. Sympathin from smooth muscle does not seem to be influenced in this way. It is true, however, that the substance given off from the liver is capable not only of accelerating the heart but can cause also a rise of blood pressure and a contraction of the denervated nictitating membrane. Further experimenting may prove that the liver gives off sympathin as do other organs innervated by the sympathetic but that the amount given off is affected by the character of the food which has been eaten. Indeed, it is not beyond the bounds of possibility that the liver is a place where sympathin and adrenin are prepared from a simple amino acid closely related to them in chemical structure.

The evidence that adrenin and sympathin are similar if not identical substances has an interesting bearing on an idea expressed by Elliott (21) about twenty-five years ago. Struck by the fact that adrenin has its usual influence on smooth muscle cells though the sympathetic nerve supply has been cut and has degenerated, he concluded that adrenin does not act by exciting a structure derived from, or dependent for its existence on, the peripheral neurone; and he suggested that the muscle cell in response to sympathetic connections develops a local mechanism for receiving and transforming the nerve impulses and that adrenin might be the chemical stimulant that is liberated whenever the nerve impulses arrive at the periphery.

According to this idea, adrenin from the adrenal medulla would mimic the action of sympathetic impulses because it is the same substance as that which mediates the action of nerve impulses on the special mechanism (contraction or inhibition) in the effector cell. Indeed, Elliott in his classic paper of 1905 (22) figures such a cell where the contractile structure lies in a less differentiated mass of cell protoplasm in which, it is assumed, the substance is set free by the nerve impulses. Only a small extension of Elliott's hypothesis is needed to explain the phenomenon of humoral transmission of sympathetic impulses from smooth muscle to other structures under sympathetic control—so much local sympathin might be liberated by the impulses that it overflows the cell boundaries and enters the blood stream. Once in the stream it has the same effects as adrenin.

An alternative idea regarding the relation of nerve ending to effector organ has been suggested by Parker (23). He points out that the shrimp, *Palaemonetes,* which has red-yellow chromatophores, becomes dark when placed in a black dish containing sea water, because of the expansion of these organs, and turns light again if placed in a white dish, because of their contraction. The color changes are dependent upon the animal's eyes, only one of which need be functional. If both eyes are blinded the chromatophores expand and thereafter no change occurs, irrespective of the illumination of the background. Older workers assumed that these changes were brought about by nervous influences affecting the expansile and contractile structures, but Perkins (24) showed that removal of nerve connections did not affect the changes, whereas interference with the blood supply would cause the chromatophores, if not already expanded, to pass quickly into that state and remain so indefinitely. Obviously some agent carried in the blood caused the contraction. Perkins injected watery extracts from many organs of the body of the shrimp, but without success. Finally he discovered that extracts of the eye stalks, injected into *Palaemonetes* that had been blinded and that were therefore dark from expanded chromatophores, caused these cells to become contracted and remain so for about a day. The conclusion was drawn that the eye stalks, when stimulated by light from a white background, produce a substance which passes into the blood and thus excites all over the body a contraction of the chromatophores. These observations are typical of similar observations made on other crustacea—indeed, extracts have been found which will cause both contraction and expansion of the pigment cells. These responses of sensitive effectors to the stimulation of the eye in invertebrates Parker likens to similar phenomena in fishes, amphibians and reptiles. In amphibians, for example, according to Hogben and Slome (25), the chromatophores are controlled not only by nerves but also through the secretion of pituitrin; in these animals, however, as in the crustacea, the eye is essential to the action of the controlling substance. In fishes and reptiles the chromatophores appear to be dominated directly by nerves, but adrenin has the same effect as the nerve impulses, *i.e.,* it causes contraction of the pigment cells. In view of the fact that the chemical sub-

stances which influence chromatophores are of nervous origin—from the eye stalk, for example, in *Palaemonetes,* and from the adrenal medulla and the posterior lobe of the pituitary body—Parker suggests that the agent which acts when nerve impulses arrive at an effector cell is a *secretion of the nerve terminals* and not, therefore, a derivative of the cell itself. In other words, the humoral intermediary between the nerve impulse and the responsive terminal organ lies within the cell, according to Elliott, but is produced by the nerve terminal and affects the cell by entering it, according to Parker.

At present, I do not know of any evidence which will clearly determine which of these two views is correct. The fact that the best known humoral mediators of nerve impulses come from cells of nervous origin favors, by analogy, the production of a substance by the nerves themselves at their terminations. Sympathin might thus be derived from the sympathetic nerve endings. On the other hand, the absence of response of smooth muscle to adrenin when such muscle does not have sympathetic connections (22) indicates that there is a peculiar relation of the sympathetic fibres to the muscle structure and function. Elliott's notion that the nerve and the muscle form a continuous mass and that somehow the fusion of sympathetic axones with smooth muscle gives to the muscle the peculiar property of responding to adrenin, might be interpreted as an endowment of the smooth muscle cell with some of the properties of the nervous structure which Parker postulates. It seems almost impossible that sufficient substance could be produced by the minute nerve terminals to affect not only the immediate cells with which they are in contact, but also other structures distributed widely over the body; as, for example, the fact that sympathin from a small patch of hair on the back acts effectively, after being diluted in the blood stream, on the denervated heart or nictitating membrane. On the other hand, if the substance is produced inside of relatively large smooth muscle cells and there acts on the contractile mechanism, it might readily be produced in such abundance as to escape and have distant effects. Unfortunately the action of drugs does not definitely discriminate between these two views of the humoral mediation of nerve impulses. Ergotamine, for example, will block nerve impulses and also the action of adrenin. It will not block the production of sympathin by nerve impulses. This fact could be accounted for on Parker's view by assuming that ergotamine prevents the passage of adrenin into the responsive cell; it could equally well be explained on Elliott's view by assuming that it prevents the action of adrenin or the locally liberated sympathin on the contractile structure inside the cellular organization. For the present we must leave this difference of view unsettled.

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